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Alternative Culture Media for Cultivation of Bacteria

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Abstract: ***Microorganisms can be grown using artificial media. Nutrient agar is a common medium used to grow bacteria in laboratories. This study was carried out to find an alternate and cheap media to cultivate bacteria. The stock obtained by soaking pulses was used as an alternate to nutrient broth. The finely ground powder of the pulses along with agar served as an alternate to nutrient agar for the cultivation of bacteria. Both the formulated media supported the growth of test organisms as the ingredients are rich in protein and carbohydrate, essential for the growth of any organism.***

Keywords: Cultivate, Media, Alternate, Pulses

1. Introduction

Food is any substance consumed to provide nutritional support for an organism. It is usually of plant or animal origin, and contains essential nutrients such as carbohydrates, fats, vitamins, proteins or minerals. Preparation of suitable media is a prerequisite to study micro-organisms. A medium is an environment which supplies the ingredients necessary for the growth of an organism. Various kinds of media have been prepared in the laboratory to isolate, grow and identify an organism. Depending on the need, to isolate and identify an organism from a particular sample or environment, different kinds of media are formulated. Different micro-organisms grow in different environments and have variety of growth requirements like nutrients, pH, osmotic conditions and temperature. Nutrient agar is a common medium used to grow bacteria in laboratories. This is a basic medium composed of a simple peptone and a beef extract. As the readily available culture media are expensive, there is a need to find alternative media for cultivating microorganisms in laboratories with less facility.

The current limitations of cultivation of microbes in a laboratory need to be addressed by formulation of a new media. Research reports indicate that alternative culture media containing cereals resulted in better growth of microorganisms when compared to the standards (Adesemoye,A.O. and Adedire, C.O., 2005). Vegetables can also be used as alternate nutrient source for microbial growth. The vegetable media is found to be a good and cheap media material for the isolation and cultivation of both bacteria and fungi (Deivanayaki, M. and Antony ruthayaraj, P., 2012). Legume seeds, rich in proteins can act as an alternative culture media for bacterial growth (Ravimannan, N. *et al*., 2012). Vegetable waste as a culture media supported the growth of bacteria, fungi, and yeast and can be used as alternative microbiological media for laboratory and industry (Dr. Chanda V. Berde and Dr. Vikrant B. Berde, 2015). Avocado seeds represent a cost effective material for producing a sustainable culture medium for bacterial growth of *E.coli* and other strains of interest in biotechnological processes (Olivia Tzintzun, *et al*., 2016). Considering low cost , watermelon agar medium, muskmelon agar medium can be considered as an alternative for the present day conventional medias for the growth of microbes (Vuyyuru Vishwanadh reddy, *et al*., 2017). Considering their nutritional values, the kitchen wastes can be utilized for production of alternative culture media (Arati Kadam, *et al*., 2018). Availability of low cost media rich in nutrients, giving comparative results is the need of the day. Recent research has been focused on finding alternative culture media for the cultivation of bacteria.

2. Materials and Methods

Collection of samples: Black gram, yellow gram, green gram and horse gram were purchased, cleaned and store in air tight containers for further use.

Test organisms used: *Staphylococcus aureus* and *Escherichia coli*.

Formulation of alternative media:

- Preparation of broth: 2 grams of each sample was weighed and soaked in 20 ml of the distilled water for overnight. The soaked samples were filtered to obtain the broth. The broth was sterilized in an autoclave at 121°C for 20 minutes under 15 psi pressure and were poured into sterile test tubes separately.
- Solid media formulation: The samples were finely powdered separately using electric blender and sieved. The powder was stored separately in sterile containers until its use. 3g from each protein source was taken and mixed with 3 grams of agar (HIMEDIA) and dissolved in 100ml distilled water. The pH of the media was measured and adjusted to 7 ± 0.2. The dissolved media was sterilized in an autoclave at 121°C for 20 minutes under 15 psi pressure and were poured into sterile Petri dishes separately.

Preparation of fresh microbial culture: Bacterial cultures used for analysis were *Staphylococcus aureus* and *Escherichia coli*. These bacteria were inoculated in the freshly prepared nutrient agar and nutrient broth medium from the original stock culture separately. The cultures were then incubated at 37°C for 24 hours.

Microbial inoculation into alternative media: The young cultures of the test bacteria *Staphylococcus aureus* and *Escherichia coli* were inoculated in each of the alternative culture medium (broth and agar media). Then all the plates and test tubes were incubated at 37°C for 48 hours.

Analysis of bacterial growth in formulated media: After incubation the turbidity of the samples was measured at 600nm which corresponds to the growth of the bacteria in

broth culture. The agar plates were observed for the degree of growth in terms of number of colonies.

Estimation of protein and carbohydrates: Protein content of the formulated broth was estimated by Folin Lowry's method while carbohydrate content was analysed by DNSA method.

3. Results and Discussion

Alternative media supported the growth of test organisms, *Staphylococcus aureus* and *Escherichia coli*. There was no significant variation in the colony morphology. Figures 1, 2 and 3 illustrate the colony morphology of the test organisms.

Figure 1: Growth of *Staphylococcus aureus* on nutrient agar

Figure 2: Growth of *Staphylococcus aureus* on alternate media using yellow gram

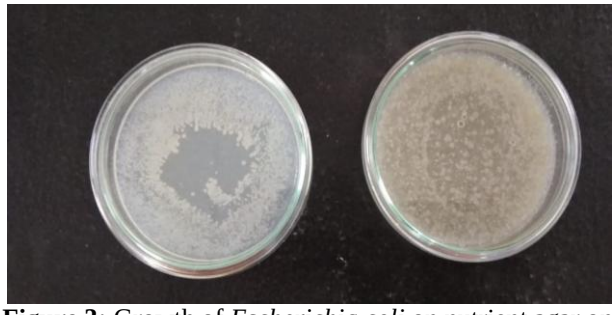

Figure 3: Growth of *Escherichia coli* on nutrient agar on left side and on alternate media on right side

Turbidometric measurement of growth of the test organisms has been shown in table 1. Maximum and minimum growth was observed with yellow gram broth and green gram broth respectively. When compared to the control, that is nutrient broth, all the alternative formulations showed better growth. So these formulations can be used as an alternate to nutrient broth for the growth of bacteria.

Table 1: Turbidometric measurement of growth of the test organisms

S. No.	Sample	Optical density (at 600nm) *Staphylococcus aureus*	Optical density (at 600nm) *Escherichia coli*
1	Black gram	1.25	0.62
2	Green gram	0.69	0.47
3	Yellow gram	1.35	1.02
4	Horse gram	0.82	0.47
5	Nutrient broth (control)	0.57	0.77

So these formulations can be used as an alternate to nutrient broth for the growth of bacteria. The protein and sugar content was estimated by standard methods and has been presented in table 2.

Table 2: Concentration of protein and sugar in alternative media

S/ No.	Formulation	Protein content (µg/ml)	Sugar content (mg/ml)
1	Black gram broth	201.0	31
2	Green gram broth	195.0	24
3	Yellow gram broth	237.5	39
4	Horse gram broth	235.5	21

Thus, being rich in these components, the media is able to support growth of microorganisms. Therefore by using the natural sources like cereals and pulses (instead of commercial culture media) for the cultivation of bacteria, the economy of the laboratories in colleges or universities can be reduced.

4. Conclusion

Based on the findings of this study, it is concluded that alternative media supported the growth of both the test organisms *Staphylococcus aureus* and *Escherichia coli*. Proteins and carbohydrates are very essential nutrients for the growth of any organism. The alternative media which can be prepared very easily using different pulses is rich in these components and therefore can be used as an alternate to nutrient broth or nutrient agar. Alternative media could be used as cheap media for routine experiments in laboratory.

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